

11 β -Hydroxysteroid Dehydrogenase and Glucocorticoid Receptor Messenger RNA Expression in Porcine Placentae: Effects of Stage of Gestation, Breed, and Uterine Environment¹

H.G. Klemcke,^{2,4} R. Sampath Kumar,^{3,5} K. Yang,⁵ J.L. Vallet,⁴ and R.K. Christenson⁴

USDA-ARS,⁴ Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933

The Lawson Research Institute,⁵ St. Joseph's Hospital, Departments of Obstetrics and Gynecology and Physiology, University of Western Ontario, London, Ontario, Canada N6A 4V2

ABSTRACT

Glucocorticoids are known to influence many aspects of prenatal development. Three important regulators of glucocorticoid actions at the cellular level are the enzymes 11 β -hydroxysteroid dehydrogenase type 1 (11 β HSD-1), 11 β -hydroxysteroid dehydrogenase type 2 (11 β HSD-2), and glucocorticoid receptors (GR). The present study was conducted to determine the presence of these regulators in porcine placentae during early gestation (Days 24–40; term = 114 days) and to examine the influence of breed and uterine environment. Three pig models differing in uterine environment as reflected by embryonic survival from Days 24 to 40 were used: intact white cross-bred gilts (WC-INT); white cross-bred gilts that had been unilaterally hysterectomized-ovariectomized before puberty (WC-UHO); and intact Meishan gilts (ME). Porcine-specific partial cDNAs for 11 β HSD-1 and 11 β HSD-2 and a cRNA for GR α were developed and used to produce ³²P-labeled probes for Northern blot analyses. The 11 β HSD dehydrogenase activity was measured in vitro at saturating concentrations of substrate and coenzyme. At Day 24 of gestation, 11 β HSD-2 mRNA, dehydrogenase activity, and GR mRNA were present, but 11 β HSD-1 mRNA was absent. All three mRNAs and dehydrogenase activity increased ($P < 0.01$) by Day 40. On Day 30, placental 11 β HSD-2 mRNA was decreased ($P = 0.03$) by 47% in WC-UHO versus WC-INT. Placental 11 β HSD dehydrogenase activity was 2-fold greater ($P < 0.01$) in ME versus WC-INT on Day 24 of gestation. These results demonstrate, to our knowledge for the first time, the presence of 11 β HSD-1, 11 β HSD-2, and GR mRNA as well as 11 β HSD dehydrogenase activity in the porcine placenta during early pregnancy. Moreover, a role for glucocorticoids in porcine embryonic development is suggested.

conceptus, cortisol, developmental biology, embryo, glucocorticoid receptor

INTRODUCTION

Multiple mechanisms exist for regulating cellular actions of glucocorticoids such as cortisol. One such mechanism is

the enzyme 11 β -hydroxysteroid dehydrogenase (11 β HSD) that reversibly converts biologically active cortisol to inactive cortisone. Two predominant 11 β HSD isoforms exist: 11 β HSD type 1 (11 β HSD-1), which has both dehydrogenase and oxoreductase activities and uses NADP⁺ and NADPH, respectively, as coenzymes [1, 2] but usually acts as a reductase enzyme [3, 4], and 11 β HSD type 2 (11 β HSD-2), which has only dehydrogenase activity and uses NAD⁺ as a coenzyme [5]. Previous work revealed placental 11 β HSD activity in numerous species (e.g., [6–10]). We demonstrated with pigs that maternal cortisol was converted to cortisone as it traverses the porcine placenta, and indeed, maternal cortisol provided 50% of fetal cortisone during mid and late gestation [11]. We subsequently showed that this placental cortisol conversion to cortisone resulted from the presence of NAD⁺- and NADP⁺-dependent 11 β HSD dehydrogenase activities [12]. We also used placental tissue fragment cultures with endogenous levels of substrates and coenzymes to show the following at 75 days of gestation: 1) both 11 β HSD oxoreductase and dehydrogenase activities were present, 2) dehydrogenase activity was 5-fold greater than oxoreductase activity, and 3) significant positive linear associations were present between net dehydrogenase activity and fetal or placental size [13].

A second cellular mechanism that regulates biological effects of glucocorticoids is the glucocorticoid receptor (GR). In humans, the GR consists of two protein isoforms, GR α and GR β , with GR α being the biologically relevant isoform [14, 15]. The GR β isoform is unable to bind glucocorticoid hormones but may form heterodimers with GR α , thereby altering the ability of GR α to regulate transcription [14, 15]. Thus, although the presence of cortisol in porcine embryos as early as Day 24 of gestation [16] suggests that cortisol may influence early embryonic development, this is necessary—but not sufficient—evidence that cortisol is active. The GRs must also be present within embryonic and placental target tissues for cortisol to exert its effects.

Therefore, to provide further suggestive evidence for an action of glucocorticoids on both porcine placental development and function and embryonic development, the objectives of the present study were as follows: 1) to determine if placental 11 β HSD-1 and 11 β HSD-2 mRNA expression and dehydrogenase activity are present in porcine placentae during early gestation (Days 24, 30, and 40); 2) to determine if this 11 β HSD expression is influenced by breed and uterine environment; and 3) to determine if mRNA expression of GR is present in embryonic placentae at these gestational ages. Because glucocorticoids are

¹Mention of trade names is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

²Correspondence: H.G. Klemcke, U.S. Army Institute of Surgical Research, 3400 Rawley E. Chambers Ave., Fort Sam Houston, TX 78234. e-mail: harold.klemcke@cen.amedd.army.mil

³Current address: CuraGen Corporation, 16 Commercial Street, Branford, CT 06405.

Received: 18 April 2003.

First decision: 13 May 2003.

Accepted: 6 August 2003.

© 2003 by the Society for the Study of Reproduction, Inc.

ISSN: 0006-3363. <http://www.biolreprod.org>

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 01 DEC 2003		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE 11β-hydroxysteroid dehydrogenase and glucocorticoid receptor mRNA expression in porcine placentae: Effects of stage of gestation, breed, and uterine environment.				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Klemcke H. G., Sampath-Kumar R., Yang K., Vallet J. L., Christenson R. K.,				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX 78232				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT SAR	18. NUMBER OF PAGES 6	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

known to influence placental and fetal size as well as fetal survival in other species (e.g., [17, 18]), it was considered to be of interest to determine if these cellular mechanisms regulating cortisol actions might be altered by breed or uterine environments associated with altered fetal size and survival.

MATERIALS AND METHODS

Animals, Experimental Design, and Tissue Collections

White crossbred (WC) gilts remained intact (INT; $n = 17$) or were unilaterally hysterectomized and ovariectomized (UHO; $n = 18$) at 160 days of age as previously described [19]. Chinese Meishan gilts (ME; $n = 18$), which are known for their increased litter size [20], remained intact. Gilts were randomly assigned to be sampled at Day 24, 30, or 40 of gestation and were bred as previously described for this study [21]. On the appropriate day of gestation, gilts were slaughtered in the U.S. Department of Agriculture Meat Animal Research Center abattoir. Reproductive tracts were removed immediately, and placental samples were obtained as rapidly as possible. Tissue was rapidly frozen in liquid nitrogen and stored frozen at -80°C until used for 11 β HSD and GR analyses. All procedures involving use of animals were reviewed and approved by the Institutional Animal Care and Use Committee.

Assay Procedures

11 β HSD dehydrogenase activity. Detailed assay procedures and validation for placental 11 β HSD dehydrogenase were previously published [12]. Enzymatic activity was measured in the presence of saturating substrate (2.5 μM unlabeled + 0.86 μCi labeled cortisol) and coenzyme (800 μM NAD $^{+}$) concentrations. Filtered placental homogenates containing protein concentrations of 450 ± 26 μg (mean \pm SEM) per 100 μl were prepared as described previously [12]. For the current studies, seven assays were conducted that each included one replicate from all treatment groups. The same nonexperimental sample served as an internal standard in each assay and provided an interassay coefficient of variation (CV) of 23.9%. All samples were assayed in triplicate (average CV of triplicate determinations = 5.23%), and specific conversion of [1,2,6,7- ^3H]cortisol (Amersham, Arlington Heights, IL) into labeled cortisone was calculated by subtracting nonspecific conversion in the presence of a heat-denatured placental preparation from total conversion. Each assay was used as a blocking factor in the statistical analysis of enzymatic data. One assay (block) was repeated once, and the average of the two assays for each sample was used in subsequent analyses.

Numerous attempts were made to validate procedures for measurement of 11 β HSD oxoreductase activity in homogenates prepared from frozen tissues. This assay made use of [^3H]cortisone and the coenzyme NADPH. Many steps in the validation (linearity with time, 0–30 min; linearity with protein concentration, 230–950 μg protein; optimum pH, 6.0) and determination of saturating coenzyme ($K_m = 18.9$ μM) concentrations were accomplished. However, repeatability among experimental assays proved to be exceptionally poor, casting doubt on the reliability of the data and, perhaps, reflecting problems that other investigators have experienced using frozen and stored or homogenized tissues [9, 22]. Hence, no data are presented for 11 β HSD oxoreductase activity.

RNA isolation and Northern blot analyses. Total RNA was isolated from tissues using RNeasy Kits (Qiagen, Chatsworth, CA). Placental RNA was loaded onto denaturing MOPS (3-[N-Morpholino] propanesulfonic acid) 1.25% agarose/formaldehyde gels, and electrophoresis was conducted. Subsequently RNA was transferred to nylon membranes (Hybond-N; Amersham) via capillary blotting and was fixed to the membrane using ultraviolet (UV) cross-linking (UV Stratalinker 2400; Stratagene, La Jolla, CA). Porcine cDNA for 11 β HSD-1 was cloned from adult porcine liver, and cDNA for 11 β HSD-2 was cloned from adult kidney. Both cDNAs were obtained by a modified 3'-rapid amplification of cDNA ends and cloned into a pBluescript KS vector (Stratagene). Briefly, first-strand cDNAs were synthesized using an oligo-dT-adaptor primer (designed in house; 5' GTC GAC GGT ACC GAT ATC T17 3') in a total volume of 20 μl . An aliquot (2 μl) was then subjected to a standard polymerase chain reaction (PCR; 94 $^{\circ}\text{C}$, 55 sec; 50 $^{\circ}\text{C}$, 55 sec; 72 $^{\circ}\text{C}$, 2 min; 30 cycles) by using the adaptor primer and a gene-specific primer (5' GGG GGG TAC CCG GGT AGA AAG CTC TGT AGG 3') that corresponds to nucleotides 1–20 in the ovine 11 β HSD-1 cDNA [23] and the primer 5' CTG AAG CTG CTG CAG ATG GA 3' that corresponds to nucleotides 405–424 in the murine 11 β HSD-2 cDNA [24]. These cDNAs were sub-

sequently sequenced, determined to be more than 70% homologous with other mammalian counterparts, and submitted to GenBank (11 β HSD-1, accession no. AF 414124, 1348 base pairs [bp]; 11 β HSD-2, accession number AF 414125, 1304 bp). Shorter cDNAs to be used for Northern blot analyses were generated from these larger cDNAs using, for 11 β HSD-1, the forward-primer 5' TTC TGG GGA TCT TCT TGG C 3' and the reverse-primer 5' AGT GGA TTC GCC ATT TTC C 3' and, for 11 β HSD-2, the forward-primer 5' GCC AGC AGA CAT TAG CCG 3' and the reverse-primer 5' AAG TAC ATG AGC CCC AGG C 3'. These primer pairs generated a cDNA for 11 β HSD-1 that contained a 505-bp fragment that was 88–90% homologous with other species. Primer pairs for 11 β HSD-2 generated a cDNA that contained a 624-bp fragment and was 83–87% homologous with other species. These cDNAs for 11 β HSD-1 and 11 β HSD-2 had no sequence similarity with the other 11 β HSD isoform.

For the porcine GR, total RNA was isolated from female pig liver. Reverse transcription was then conducted as previously described [25] using random nonamer primers (Amersham) to produce cDNA. To amplify the GR, specific cDNA primers were designed for regions of the human GR α 3'-untranslated region [14]. These primers (forward primer, 5' TTG GTG CTT CTA ACC TGA TGG 3'; reverse primer, 5' GAT GGG AAT GTG AAA ATG GG 3') produced a 491-bp cDNA that would be specific for GR α [14] if the porcine GR is comparable to the human GR. This sequence was found to be 89.6% homologous with human GR 9 α (U80946 and X03225) and was submitted to GenBank (accession no. AY007222). Porcine cDNA for porcine β -actin was generated, and cDNA were cloned and sequenced as previously described [25].

For Northern blot analyses, ^{32}P -labelled cDNAs were prepared using PCR procedures, [^{32}P]dCTP (Dupont New England Nuclear, Wilmington, DE), and the above-noted primers specific for 11 β HSD and β -actin transcripts. For Northern blot analyses of GR, and for β -actin in the same Northern blot membrane preparations, cRNAs were prepared, and procedures detailed in Ambion's MAXIScript in vitro transcription kit (Ambion, Austin, TX) and [α - ^{32}P] UTP (Amersham) were used for preparation of ^{32}P -labelled cRNA probes.

Northern blot analyses using these labeled cDNAs and cRNAs were conducted essentially as previously described for erythropoietin mRNA [25]. To insure that variability among processing operations was evenly distributed across all treatments, RNA from each treatment was equally represented on each gel/membrane, and membranes were used as a blocking factor in statistical analyses. Densitometric measures were conducted with a ChemImager (Alpha Innotech Corp., San Leandro, CA), or with Epi Chemi Darkroom (UVP, Inc., Upland, CA) used in conjunction with the NIH Image program (National Institutes of Health, Research Services Branch, Bethesda, MD. Website at http://rsb.info.nih.gov/nih_image/).

Statistical Analyses

Data for mRNA and enzymatic activity were analyzed using the Statistical Analysis System [26]. All data were examined for normality of distribution (PROC UNIVARIATE NORMAL) and homogeneity of variance (F_{max} test) and were transformed to a log or square root function when necessary to meet assumptions of analysis of variance. Enzymatic activity (mean of replicate analyses for each sample) was then analyzed using PROC MIXED and two-way analysis of covariance (ANOCVA) with treatments and day of gestation as main factors and assay as a random factor. Activity of the internal standard in each assay served as the covariate. All data for mRNA bands of interest were also analyzed using PROC MIXED and two-way ANOCVA with treatments and day of gestation as main factors and gel as a random factor. Each mRNA of interest was adjusted using ANOCVA and expression of β -actin in the same lane as a covariate to account for potential differences in lane loading and membrane transfer. Comparisons of individual treatment means were made by *a priori* orthogonal contrasts. The least-squares mean \pm SEM of original data are presented. A probability level of $P \leq 0.05$ was considered to be significant.

RESULTS

Fetal and placental data for the present study have been reported elsewhere in detail [21] and will not be repeated here. A single band of 11 β HSD-1 mRNA was observed at approximately 1.9 kilobases (kb) (Fig. 1A). Under conditions of the present study, placental mRNA expression of 11 β HSD-1 was undetectable on Day 24 of gestation (Fig. 1A), with the exception of one Meishan fetus. Hence, data for Day 24 were not included in the statistical analyses (Fig.

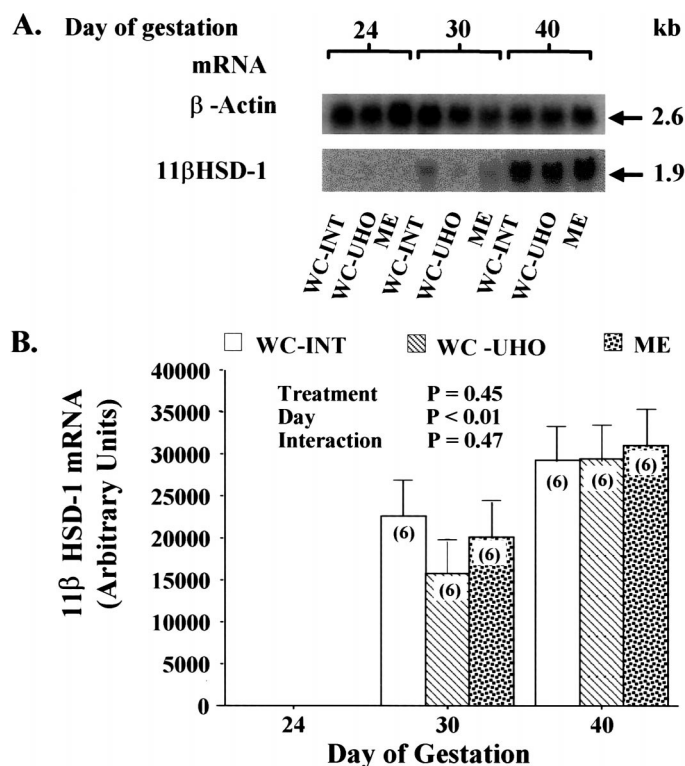


FIG. 1. A) Northern blot analysis of placental 11βHSD-1 mRNA expression at Days 24, 30, and 40 of gestation. Each lane contained 10 μg of total RNA. B) Results of densitometric analysis (ChemImager; Alpha Innotech Corp., San Leandro, CA) of steady-state levels of placental 11βHSD-1 mRNA. Values for 11βHSD-1 were adjusted by analysis of covariance to those of β-actin for the same sample. Results represent the least-squares mean + SEM of the number of replicates indicated by numbers within bars. WC-INT, intact white crossbred gilts; WC-UHO, unilaterally-hysterectomized-ovariectomized white crossbred gilts; ME, intact Meishan gilts.

1B). The 11βHSD-1 mRNA expression was modestly present by Day 30 (Fig. 1). Between Days 30 and 40, a 53% increase ($P < 0.01$) was observed in the expression of placental 11βHSD-1 mRNA in all pig models (Fig. 1). No differences ($P = 0.45$) were observed among the three treatments (pig models).

On the contrary, 11βHSD-2 mRNA expression was already readily apparent on Day 24 (Fig. 2). In contrast to 11βHSD-1, 11βHSD-2 mRNA had four distinct bands (Fig. 2A). A decidedly major band (band A) was present at approximately 2.3 kb, with more minor bands occurring at approximately 1.8, 1.5, and 1.2 kb. Statistical evaluations were conducted on each individual band and on the sum of the densitometric measurements for all bands. Analysis for the major band is presented (Fig. 2B). For this major band, ANOCVA did not reveal any treatment effects ($P = 0.51$) or treatment × day interactions ($P = 0.10$), but it did demonstrate marked day effects ($P = 0.01$) (Fig. 2B). These day effects represent an increase in mRNA expression across all treatments on Day 40 compared with Day 30 ($P = 0.005$). A priori orthogonal contrasts indicate that on Day 30, 11βHSD-2 mRNA expression is less in WC-UHO pigs than in WC-INT pigs ($P = 0.03$). When the sum of bands A through D is evaluated, the interpretation of results is the same (data not shown).

Placental 11βHSD dehydrogenase activity displayed marked, day-dependent increases in activity between Days 30 and 40 of gestation ($P = < 0.01$) (Fig. 3). On Day 24

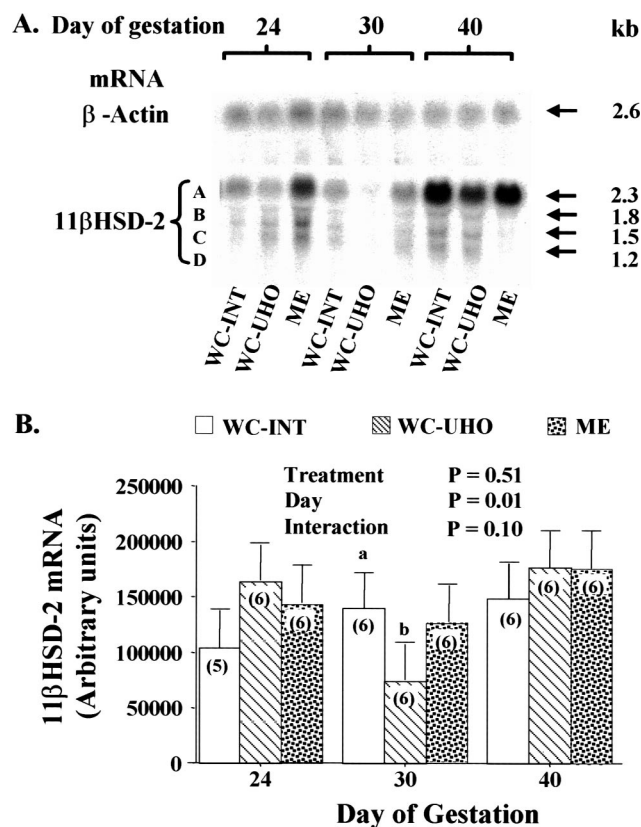


FIG. 2. A) Northern blot analysis of placental 11βHSD-2 mRNA expression at Days 24, 30, and 40 of gestation. Each lane contained 10 μg of total RNA. B) Results of densitometric analysis (ChemImager; Alpha Innotech Corp., San Leandro, CA) of steady-state levels of placental 11βHSD-2 mRNA for only the major band in lane A. Values for 11βHSD-2 were adjusted by analysis of covariance to those of β-actin for the same sample. Results represent the least-squares mean + SEM of the number of replicates indicated by numbers within bars. Statistics were conducted on log₁₀-transformed data. A priori orthogonal contrasts indicate that on Day 30, 11βHSD-2 mRNA expression is less in WC-UHO pigs than in WC-INT pigs ($P = 0.03$, a vs. b). WC-INT, intact white crossbred gilts; WC-UHO, unilaterally-hysterectomized-ovariectomized white crossbred gilts; ME, intact Meishan gilts.

(using an a priori orthogonal contrast), 11βHSD dehydrogenase activity in ME placentae was greater than in WC-INT placentae on the same days ($P < 0.037$). Although 11βHSD-2 mRNA and dehydrogenase activity increased in parallel between Days 30 and 40, ANOCVA procedures using 11βHSD-2 mRNA expression as a covariate did not detect a significant linear relationship between dehydrogenase activity and mRNA expression as measured by the most prominent band or by the sum of all bands ($P > 0.47$).

Expression of mRNA for GR was detected in placentae at each stage of gestation (Fig. 4). Labeled cRNA hybridized with a mRNA of approximately 8.8 kb. An increase ($P = 0.013$) was observed in GR expression with age that was independent of treatment ($P = 0.83$) (Fig. 4B) and that represented a 37% increase ($P < 0.01$) between Days 30 and 40. Placental β-actin expression was not affected by treatment ($P > 0.22$).

DISCUSSION

In the present study, the findings of primary importance are as follows: 1) 11βHSD-2 mRNA expression and enzymatic activity are present within porcine placentae as early as Day 24 of gestation; 2) placental 11βHSD-1 mRNA

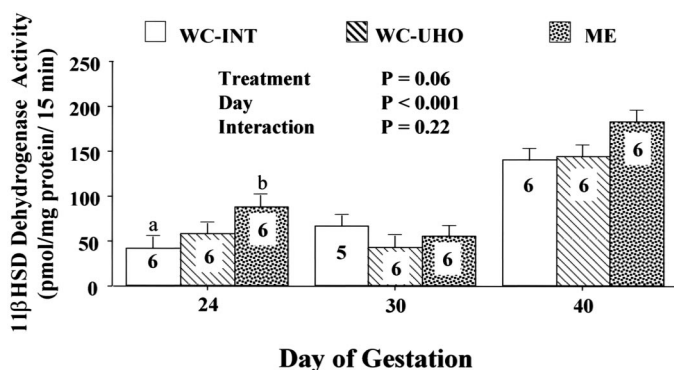


FIG. 3. Effects of breed, uterine environment, and gestational age on placental NAD⁺-dependent 11βHSD dehydrogenase activity under optimal in vitro conditions of pH (9.0), coenzyme concentration (800 μM), substrate concentration (2.5 μM plus 0.86 μCi [³H]cortisol), time (15 min), and temperature (37°C). Each bar represents the mean + SEM of the number of pigs in parentheses. Statistics were conducted on square-root-transformed data. A priori orthogonal contrasts show that this enzymatic activity was greater in Meishan placenta compared with WC-INT placenta on Day 24 ($P < 0.037$, a vs. b). WC-INT, intact white crossbred gilts; WC-UHO, unilaterally-hysterectomized-ovariectomized white crossbred gilts; ME, intact Meishan gilts.

expression was not detected on Day 24 of gestation; 3) both 11βHSD-1 and 11βHSD-2 mRNA expression as well as 11βHSD dehydrogenase activity increased between Days 30 and 40 of gestation; 4) placental mRNA expression of GR was present as early as Day 24 of gestation, and this expression increased with gestation; 5) 11βHSD-2 mRNA expression on Day 30 of gestation decreased in embryos of WC-UHO gilts relative to WC-INT gilts; and 6) on Day 24 of gestation, placental 11βHSD dehydrogenase activity was greater in Meishan compared with WC-INT gilts.

For all species in which detailed studies have been conducted, 11βHSD-2 functions primarily as a dehydrogenase (converts active cortisol to inactive cortisone) [27], and 11βHSD-1, although capable of oxidative and reductive activity, functions primarily as a reductase (converts cortisone to cortisol) [27]. In the present study, we cannot say with certainty that dehydrogenase activity represents only 11βHSD-2 function. However, the presence of 11βHSD-2 mRNA expression and assumed (dehydrogenase) biological activity at Day 24, along with the absence of 11βHSD-1 mRNA expression, suggest an importance for lower active glucocorticoid concentrations in both placentae and embryos. This importance is further suggested by the decline in placental 11βHSD-2 mRNA expression on Day 30 of gestation in embryos of WC-UHO gilts relative to WC-INT gilts. It was previously shown that decreased embryonic survival occurred in the crowded uterine environment on Day 40 compared with Day 30, although placental weights were not compromised [21]. However, more recent results demonstrated 38% decreases ($P = 0.01$) in placental weight on Day 35 in WC-UHO gilts [25].

The cause of decreased 11βHSD-2 mRNA is unknown. Of the mRNA measured (β-actin, GR, 11βHSD-1, and 11βHSD-2), only 11βHSD-2 mRNA expression differed between WC-UHO and WC-INT gilts. This suggests a specificity of response in terms of at least these limited numbers of mRNAs. Hence, lower 11βHSD-2 at Day 30 may be associated with an increased number of compromised embryos that will die by Day 40. Whether altered placental 11βHSD expression is one cause of subsequent mortality, merely symptomatic of embryonic morbidity, or

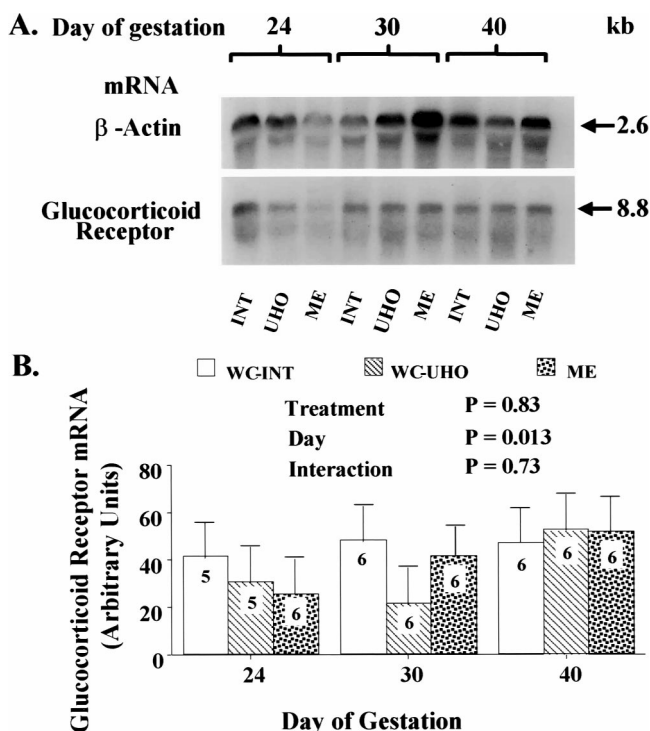


FIG. 4. A) Northern blot analysis of placental GR mRNA expression at Days 24, 30, and 40 of gestation. Each lane contained 30 μg of total RNA. B) Results of densitometric analysis (Epi Chemi Darkroom; UVP, Inc., Upland, CA) of steady-state levels of placental GR mRNA expression. Values for GR were adjusted by analysis of covariance to those of β-actin for the same sample. Results represent the least-squares mean + SEM of the number of replicates indicated by numbers within bars. Statistics were conducted on log₁₀-transformed data. WC-INT, intact white crossbred gilts; WC-UHO, unilaterally-hysterectomized-ovariectomized white crossbred gilts; ME, intact Meishan gilts.

unrelated to mortality and morbidity, however, remains to be determined. The 11βHSD dehydrogenase activity was not affected by the crowded uterine environment on Day 30, suggesting different sensitivities of mRNA and active protein to uterine crowding and associated biological alterations. Depending on the half-life of the 11βHSD-2 protein, reduced mRNA expression would be expected to precede a subsequently lowered activity. An alternative hypothesis would be that 11βHSD-1 protein is partially contributing to measured dehydrogenase activity in addition to its reductase activity. However, placental 11βHSD-1 dehydrogenase activity is NADP⁺-dependent [10, 12, 28], and in the present assays, NAD⁺ was used as a coenzyme. Furthermore, although intraplacental concentrations of cortisol substrate are not known, plasma cortisol concentrations on Days 16–18 of pregnancy vary from 10 to 38 ng/ml [29]. Embryonic cortisol concentrations on Day 25 are quite low (~1.5 ng/mg DNA, or <10 ng cortisol/g tissue [17]). Such cortisol concentrations are incompatible with the previously measured K_m of placental NADP⁺-dependent dehydrogenase activity (849 nM [12]). It is unlikely, therefore, that placental 11βHSD-1 provides a substantial contribution to either measured or actual dehydrogenase activity at this stage.

It is also of interest to note the increased placental 11βHSD dehydrogenase activity in Meishan versus WC-INT gilts. This is additional evidence for differences in aspects of glucocorticoid concentrations or metabolism in Meishan pigs during gestation [30] that could influence fe-

tal development and survival. This finding is also contrary to what one might anticipate if glucocorticoids were the primary factor regulating fetal size; that is, with higher placental 11 β HSD-2, one would expect lower fetal cortisol and larger fetal weights [27, 31]. This might be especially true at Day 24, when cortisol impinging on the embryo is predominantly of maternal origin [16, 32]. However, many factors regulate fetal size (e.g., [33, 34]). Additionally, our interpretation of the potential effects of glucocorticoids on fetal size and the regulation of these effects by placental 11 β HSD and GR are confounded in the present study by an absence of information on embryonic glucocorticoid regulatory mechanisms.

The present study demonstrates the presence in porcine placentae of multiple transcripts of 11 β HSD-2 mRNA expression. The most prominent band occurred at approximately 2.3 kb. In mouse kidney and colon, multiple 11 β HSD-2 mRNA transcripts were evident at approximately 5 and 2.0 kb [24]. In term human placentae, 11 β HSD-2 mRNA was present at 1.9 and 4.0 kb [35]. Hence, precedents exist for multiple 11 β HSD-2 transcripts. However, in other studies, only single transcripts of 11 β HSD-2 mRNA were observed [36–38]. The biological relevance of these various mRNA species remains to be determined. For example, which bands are translated into active protein is not known, although it might be presumed that the major band (2.3 kb) represents the mRNA that contributes most to active protein. Mechanisms contributing to the various bands may represent alternative cleavage and polyadenylation patterns, alternative splicing of transcripts from a single gene, or transcription from more than one gene. The specific mechanism was not determined, but to date, evidence exists for only one gene encoding 11 β HSD-2 in human tissues [39].

Expression of 11 β HSD-1 mRNA was almost completely absent in Day 24 placentae with the amount of total RNA (10 μ g) used. Such absence on Day 24 again suggests an additional mechanism by the developing placenta to minimize exposure of the placenta and embryo to biologically active glucocorticoids, because 11 β HSD-1 may serve to amplify glucocorticoid action at the cellular level [40].

Day 24 of gestation—relative to term in pigs (114 days; 21% or 0.21 of gestation completed)—represents the earliest measures of placental 11 β HSD mRNA expression in any species. Consequently, it is difficult to compare the relative onset of mRNA expression of these enzymes in pigs with those of other species. However, in human placenta (8–12 wk, or ~0.21–0.32 of gestation), there was a 4% conversion of cortisone to cortisol (reductase activity), but a 79% conversion of cortisol to cortisone (dehydrogenase activity [41]). In baboon placentae at early (0.33) gestation, mRNA and protein expression for both isozymes were present, and both increased with advancing gestation [38]. However, to our knowledge, earlier ages have not been measured. Therefore, the currently reported pattern of initiation of 11 β HSD mRNA expression is most nearly similar to that of enzymatic activities in humans at a comparable age (relative to term). Although, as noted above, data for oxoreductase activity were inconsistent, our measures do indicate the existence of this activity and, thereby, substantiate that translation of this 11 β HSD-1 mRNA into biologically active protein does occur.

We have previously demonstrated that intrauterine cortisol concentrations increase dramatically between Days 10 and 13 of pregnancy in gilts [42]. We have also demonstrated the presence of low cortisol concentrations in por-

cine embryos at Day 25 of gestation, with an 8-fold increase occurring by Day 35 [16]. Hence, at these early gestational stages in pigs, cortisol is present in the environment and in the embryos themselves, and it has the potential for influencing development. The currently reported early appearance of GR mRNA expression—with the caveat that it is translated into active protein—further suggests the involvement of glucocorticoids in early porcine placental development and/or function and indirectly in embryonic development. It has been previously suggested for other species that the presence of placental 11 β HSD not only reflects a regulation of transplacental passage of glucocorticoids but also a local regulation of placental glucocorticoid actions [43, 44]. Although only minimal indirect evidence exists in pigs [45], abundant direct and indirect evidence indicates a variety of effects of glucocorticoids on placental function in other species (e.g., [46–48]). Therefore, the presence of GR mRNA in the early porcine placenta is consistent with data in other species for a function of cortisol in porcine placentae.

The present study was not designed to evaluate the placental cellular location of either 11 β HSD or GR mRNA expression, as has been done for humans [38, 49, 50], baboons [49], and rats [51]. In contrast to hemochorial placentae of rat and humans, the porcine placenta is epitheliochorial in nature. Such a placenta consists of an outer layer of chorionic epithelial cells (trophectoderm) that contact the uterine endometrial epithelium, a somatic mesodermal layer, a splanchnic mesodermal layer, and finally, an inner thin layer of entodermal cells [52]. Hence, our preparations contained all these layers combined, and only by subsequent use of immunohistochemical and *in situ* hybridization techniques will it be possible to discern the cellular relationships of these enzymatic and receptor regulators of glucocorticoid actions in the porcine placenta. Nonetheless, as has been expressed for other species [44] and tissues [40], the colocalization of 11 β HSD-1, 11 β HSD-2, and GR in the porcine placenta suggests an intricate regulation of glucocorticoid action in this tissue at these early developmental stages.

In summary, these studies demonstrate the presence of 11 β HSD-1, 11 β HSD-2, and GR mRNA expression as well as 11 β HSD dehydrogenase activity in porcine placentae. Their presence suggests a role for glucocorticoids in porcine placental and embryonic development and function at these early stages of gestation.

ACKNOWLEDGMENTS

We express our appreciation to Frank Reno, David Sypherd, and Terri Alberts for technical assistance. We also express our apologies to those investigators whose publications have contributed significantly to this area of research but could not be cited because of space constraints.

REFERENCES

1. Agarwal AK, Monder C, Eckstein B, White PC. Cloning and expression of rat cDNA encoding corticosteroid 11 β -dehydrogenase. *J Biol Chem* 1989; 264:18939–18943.
2. Agarwal AK, Tusie-Luna MT, Monder C, White PC. Expression of 11 β -hydroxysteroid dehydrogenase using recombinant vaccinia virus. *Mol Endocrinol* 1990; 4:1827–1832.
3. Lakshmi V, Monder C. Purification and characterization of the corticosteroid 11 β -dehydrogenase component of the rat liver 11 β -hydroxysteroid dehydrogenase complex. *Endocrinology* 1988; 123:2390–2398.
4. Jamieson PM, Chapman KE, Edwards CR, Seckl JR. 11 β -Hydroxysteroid dehydrogenase is an exclusive 11 β -reductase in primary cul-

- tures of rat hepatocytes: effect of physicochemical and hormonal manipulations. *Endocrinology* 1995; 136:4754–4761.
5. Li KX, Obeyesekere VR, Krozowski ZS, Ferrari P. Oxoreductase and dehydrogenase activities of the human and rat 11 β -hydroxysteroid dehydrogenase type 2 enzyme. *Endocrinology* 1997; 138:2948–2952.
 6. Osinski PA. Steroid 11 β -dehydrogenase in human placenta. *Nature* 1960; 187:777.
 7. Bernal AL, Flint AP, Anderson AB, Turnbull AC. 11 β -Hydroxysteroid dehydrogenase activity (E.C. 1.1.1.146) in human placenta and decidua. *J Steroid Biochem* 1980; 13:1081–1087.
 8. Baggia S, Albrecht ED, Pepe GJ. Regulation of 11 β -hydroxysteroid dehydrogenase activity in the baboon placenta by estrogen. *Endocrinology* 1990; 126:2742–2748.
 9. Burton PJ, Waddell BJ. 11 β -Hydroxysteroid dehydrogenase in the rat placenta: developmental changes and the effects of altered glucocorticoid exposure. *J Endocrinol* 1994; 143:505–513.
 10. Yang K. Co-expression of two distinct isoforms of 11 β -hydroxysteroid dehydrogenase in the ovine placenta. *J Steroid Biochem Mol Biol* 1995; 52:337–343.
 11. Klemcke HG. Placental metabolism of cortisol at mid and late gestation in swine. *Biol Reprod* 1995; 53:1293–1301.
 12. Klemcke HG, Christenson RK. Porcine placental 11 β -hydroxysteroid dehydrogenase activity. *Biol Reprod* 1996; 55:217–223.
 13. Klemcke HG. Dehydrogenase and oxoreductase activities of porcine placental 11 β -hydroxysteroid dehydrogenase. *Life Sci* 2000; 66:1045–1052.
 14. Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, Thompson EB, Rosenfeld MG, Evans RM. Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* 1985; 318:635–641.
 15. Bamberger CM, Schulte HM, Chrousos GP. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. *Endocr Rev* 1996; 17:245–261.
 16. Klemcke HG, McGuire WJ, Christenson RK. Cortisol concentrations in early porcine embryos. *Life Sci* 1999; 64:1307–1312.
 17. Blackburn WR, Kaplan HS, McKay DG. Morphological changes in the developing rat placenta following prednisolone administration. *Am J Obstet Gynecol* 1965; 92:234–246.
 18. Roberts LG, Hendrickx AG. Hydrocortisone-induced embryotoxicity and embryonic drug disposition in H-2 congenic mice. *J Craniofac Gen Dev Biol* 1987; 7:341–356.
 19. Christenson RK, Leymaster KA, Young LD. Justification of unilateral hysterectomy-ovariectomy as a model to evaluate uterine capacity in swine. *J Anim Sci* 1987; 65:738–744.
 20. Christenson RK, Vallet JL, Leymaster KA, Young LD. Uterine function in Meishan pigs. *J Reprod Fertil Suppl* 1993; 48:279–289.
 21. Pearson PL, Klemcke HG, Christenson RK, Vallet JL. Uterine environment and breed effects on erythropoiesis and liver protein secretion in late embryonic and early fetal swine. *Biol Reprod* 1998; 58:911–918.
 22. Lopez Bernal A, Anderson AB, Turnbull AC. Cortisol: cortisone interconversion by human decidua in relation to parturition: effect of tissue manipulation on 11 β -hydroxysteroid dehydrogenase activity. *J Endocrinol* 1982; 93:141–149.
 23. Yang K, Smith CL, Dales D, Hammond GL, Challis JR. Cloning of an ovine 11 β -hydroxysteroid dehydrogenase complementary deoxyribonucleic acid: tissue and temporal distribution of its messenger ribonucleic acid during fetal and neonatal development. *Endocrinology* 1992; 131:2120–2126.
 24. Cole TJ. Cloning of the mouse 11 β -hydroxysteroid dehydrogenase type 2 gene: tissue specific expression and localization in distal convoluted tubules and collecting ducts of the kidney. *Endocrinology* 1995; 136:4693–4696.
 25. Klemcke HG, Vallet JL, Christenson RK, Pearson PL. Erythropoietin mRNA expression in pig embryos. *Anim Reprod Sci* 2001; 66:93–108.
 26. SAS Online DOC, Version 8. Cary, NC: Statistical Analysis System, Inc.; 1999.
 27. Stewart PM, Krozowski ZS. 11 β -Hydroxysteroid dehydrogenase. *Vitam Horm* 1999; 57:249–324.
 28. Lakshmi V, Nath N, Muneyyrci-Delale O. Characterization of 11 β -hydroxysteroid dehydrogenase of human placenta: evidence for the existence of two species of 11 β -hydroxysteroid dehydrogenase. *J Steroid Biochem Mol Biol* 1993; 45:391–397.
 29. Klemcke HG, Pearson PL, Vallet JL, Christenson RK. Plasma cortisol and aldosterone during early porcine pregnancy. *J Anim Sci* 1998; 76(suppl 1):241.
 30. Klemcke HG, Christenson RK. Porcine fetal and maternal adrenocorticotrophic hormone and corticosteroid concentrations during gestation and their relation to fetal size. *Biol Reprod* 1997; 57:99–106.
 31. Benediktsson R, Lindsay RS, Nobile J, Seckl JR, Edwards CRW. Glucocorticoid exposure in utero: new model for adult hypertension. *Lancet* 1993; 341:339–341.
 32. Whitehead RH. The histogenesis of the adrenal in the pig. *Am J Anat* 1903; 2:349–360.
 33. Wolpert L, Beddington R, Brokes J, Jessell T, Lawrence P, Meyerowitz E. *Principles of Development*. New York: Oxford University Press; 1998:417–441.
 34. Evain-Brion D. Hormonal regulation of fetal growth. *Horm Res* 1994; 42:207–214.
 35. Sun K, Yang K, Challis JR. Differential expression of 11 β -hydroxysteroid dehydrogenase types 1 and 2 in human placenta and fetal membranes. *J Clin Endocrinol Metab* 1997; 82:300–305.
 36. Langlois DA, Matthews SG, Yu M, Yang K. Differential expression of 11 β -hydroxysteroid dehydrogenase 1 and 2 in the developing ovine fetal liver and kidney. *J Endocrinol* 1995; 147:405–411.
 37. Campbell LE, Yu M, Yang K. Ovine 11 β -hydroxysteroid dehydrogenase type 2 gene predicts a protein distinct from that deduced by the cloned kidney cDNA at the C-terminus. *Mol Cell Endocrinol* 1996; 119:113–118.
 38. Pepe GJ, Babischkin JS, Burch MG, Leavitt MG, Albrecht ED. Developmental increase in expression of the messenger ribonucleic acid and protein levels of 11 β -hydroxysteroid dehydrogenase types 1 and 2 in the baboon placenta. *Endocrinology* 1996; 137:5678–5684.
 39. Agarwal AK, Rogerson FM, Mune T, White PC. Gene structure and chromosomal localization of the human HSD11K gene encoding the kidney (type 2) isozyme of 11 β -hydroxysteroid dehydrogenase. *Genomics* 1995; 29:195–199.
 40. Seckl JR, Walker BR. Minireview: 11 β -hydroxysteroid dehydrogenase type 1—a tissue-specific amplifier of glucocorticoid action. *Endocrinology* 2001; 142:1371–1376.
 41. Giannopoulos G, Jackson K, Tulchinsky D. Glucocorticoid metabolism in human placenta, decidua, myometrium and fetal membranes. *J Steroid Biochem* 1982; 17:371–374.
 42. Klemcke HG, Kattesh HG, Vallet JL, Roberts MP, McGuire WJ, Christenson RK. Intrauterine cortisol, aldosterone, and corticosteroid binding globulin-like activity during early porcine pregnancy and the estrous cycle. *Biol Reprod* 1998; 58:240–247.
 43. Burton PJ, Smith RE, Krozowski ZS, Waddell BJ. Zonal distribution of 11 β -hydroxysteroid dehydrogenase types 1 and 2 messenger ribonucleic acid expression in the rat placenta and decidua during late pregnancy. *Biol Reprod* 1996; 55:1023–1028.
 44. Burton PJ, Waddell BJ. Dual function of 11 β -hydroxysteroid dehydrogenase in placenta: modulating placental glucocorticoid passage and local steroid action. *Biol Reprod* 1999; 60:234–240.
 45. Gibb W, Randall GC. Prostaglandin production by porcine allantochorion in vitro: effect of cortisol infusion in vivo. *Can J Physiol Pharmacol* 1992; 70:990–995.
 46. Steele PA, Flint AP, Turnbull AC. Activity of steroid C-17,20 lyase in the ovine placenta: effect of exposure to foetal glucocorticoid. *J Endocrinol* 1976; 69:239–246.
 47. Ringler GE, Kallen CB, Strauss JF III. Regulation of human trophoblast function by glucocorticoids: dexamethasone promotes increased secretion of chorionic gonadotropin. *Endocrinology* 1989; 124:1625–1631.
 48. Jones SA, Challis JR. Steroid, corticotrophin-releasing hormone, ACTH and prostaglandin interactions in the amnion and placenta of early pregnancy in man. *J Endocrinol* 1990; 125:153–159.
 49. Pepe GJ, Burch MG, Albrecht ED. Expression of the 11 β -hydroxysteroid dehydrogenase types 1 and 2 proteins in human and baboon placental syncytiotrophoblast. *Placenta* 1999; 20:575–582.
 50. Driver PM, Kilby MD, Bujalska I, Walker EA, Hewison M, Stewart PM. Expression of 11 β -hydroxysteroid dehydrogenase isozymes and corticosteroid hormone receptors in primary cultures of human trophoblast and placental bed biopsies. *Mol Hum Reprod* 2001; 7:357–363.
 51. Waddell BJ, Benediktsson R, Brown RW, Seckl JR. Tissue-specific messenger ribonucleic acid expression of 11 β -hydroxysteroid dehydrogenase types 1 and 2 and the glucocorticoid receptor within rat placenta suggests exquisite local control of glucocorticoid action. *Endocrinology* 1998; 139:1517–1523.
 52. Patten BM. *Embryology of the Pig*, 3rd ed. New York: McGraw Hill; 1948.